ABSTRACT OF THE DISCLOSURE

Protein kinase C (PKC) has been implicated as a mediator of diabetes-induced vascular proliferation. This study investigated the regulation of PKCβ gene expression following acute glucose exposure in human vascular smooth muscle cells and in A10 cells, a rat aortic smooth muscle cell line. Western blot analysis showed that PKCβII protein levels decreased with high glucose (25mM) while PKCβI level were unaltered. PKCβ mRNA levels were depleted by 60-75% in hyperglycemic conditions. Quenching of PKCβ promoter activity by glucose suggested involvement of a carbohydrate response element in the 5' promoter region. Simultaneous cell cycle studies indicated an increase in the percentage of cells going into S phase in high glucose implying that quenching of PKCβ transcription may be related to cell cycle progression. It was demonstrated that glucose induced post-transcriptional destabilization of PKCβII message via a nuclease activity present in the cytosol. The specificity of glucose to post-transcriptionally destabilize PKCβII, but not the PKCβI, isoform was confirmed in both A10 cells and primary cultures from human aorta.

To further elucidate the intracellular signaling mechanisms, glucose analogs were used to study the pathways by which glucose acted to destabilize PKCβII mRNA. Glucose-induced destabilization of PKCβII mRNA is independent of the hexosamine or hexokinase pathways. Cycloheximide did not block destabilization of PKCβII mRNA by high glucose indicating that the process is independent of translation. Glucose may act via PKC signaling pathways and may be regulated by serine/threonine phosphorylation/dephosphorylation.

A heterologous chimeric minigene encoding PKCβII cDNA subcloned into the pβG expression vector comprising the coding sequences of β-globin genomic DNA and 3'UTR and polyadenylation site of bovine growth hormone cDNA was constructed. Half-life analysis indicated a rapid glucose-induced destabilization of β-globin mRNA in pβG-PKCβII transfected cells. Mobility shift analysis indicated the presence of a glucose-responsive instability element within the exon included in the mature PKCβII mRNA in VSMC. UV cross-linking analysis showed a small protein (~10-14 kDa) binding near a stem-loop structure within the PKCβII specific exon. This is a novel finding of a instability element

~~within the PKCβII mRNA coding region that is regulated by glucose in aorta smooth muscle cells.~~

The subject invention pertains to nucleic acid constructs for post-transcriptional control of expression of a polynucleotide encoding a protein in a eukaryotic cell, wherein the constructs include a metabolite responsive instability element such as the glucose-regulated mRNA instability element. The subject invention further pertains to host cells and vectors comprising the nucleic acid constructs of the invention, as well as probes, methods, and kits for detecting metabolite responsive instability elements or mutations thereof.